10/569,315

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

(Chapter II of the Patent Cooperation Treaty)

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference 395/46	FOR FURTHER ACTION	See Form PCT/IPEA/416
International application No. PCT/US05/18412	International filing date *(day/month/year)* 23 May 2005 (23.05.2005)	Priority date *(day/month/year)* 23 May 2004 (23.05.2004)
International Patent Classification (IPC) or national classification and IPC IPC: C07D 237/02(2006.01) USPC: 544/224		
Applicant HOUSEY PHARMACEUTICALS, INC.		

1. This report is the international preliminary examination report, established by this International Preliminary Examining Authority under Article 35 and transmitted to the applicant according to Article 36.
2. This REPORT consists of a total of 3 sheets, including this cover sheet.
3. This report is also accompanied by ANNEXES, comprising:
   a. ☑ *(sent to the applicant and to the International Bureau)* a total of 23 sheets, as follows:
      ☐ sheets of the description, claims and/or drawings which have been amended and are the basis of this report and/or sheets containing rectifications authorized by this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions).
      ☐ sheets which supersede earlier sheets, but which this Authority considers contain an amendment that goes beyond the disclosure in the international application as filed, as indicated in item 4 of Box No. I and the Supplemental Box.
   b. ☐ *(sent to the International Bureau only)* a total of (indicate type and number of electronic carrier(s)) ____, containing a sequence listing and/or tables related thereto, in electronic form only, as indicated in the Supplemental Box Relating to Sequence Listing (see Section 802 of the Administrative Instructions).
4. This report contains indications relating to the following items:
   - ☒ Box No. I Basis of the report
   - ☐ Box No. II Priority
   - ☐ Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - ☐ Box No. IV Lack of unity of invention
   - ☒ Box No. V Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
   - ☐ Box No. VI Certain documents cited
   - ☐ Box No. VII Certain defects in the international application
   - ☐ Box No. VIII Certain observations on the international application

Date of submission of the demand	Date of completion of this report
06 February 2006 (02.02.2006)	25 June 2007 (25.06.2007)
Name and mailing address of the IPEA/ US Mail Stop PCT, Attn: IPEA/US Commissioner for Patents P.O. Box 1450 Alexandria, Virginia 22313-1450 Facsimile No. (571) 273-3201	Authorized officer James O Wilson Felicia D. Roberts for Telephone No. 571-272-2908

Form PCT/IPEA/409 (cover sheet)(April 2005)

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

International application No.
PCT/US05/18412

Box No. I Basis of the report

1. With regard to the **language**, this report is based on:

☐ the international application in the language in which it was filed.

☐ a translation of the international application into _____, which is the language of a translation furnished for the purposes of:

☐ international search (under Rules 12.3 and 23.1(b))

☐ publication of the international application (under Rule 12.4(a))

☐ international preliminary examination (under Rules 55.2(a) and/or 55.3(a))

2. With regard to the **elements** of the international application, this report is based on *(replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report)*:

☐ the international application as originally filed/furnished

☒ the description:
pages 1-7,10-14,16-41,44-49,52,53,55-57,59,62-64,67-69 and 71 as originally filed/furnished
pages* 8,9,15,42,43,50,51,54,58,60,61,64,66,70 and 72-75b received by this Authority on 02 February 2006 (02.02.2006).
pages* NONE received by this Authority on _____

☒ the claims:
pages 76-88 and 91-112 as originally filed/furnished
pages* NONE as amended (together with any statement) under Article 19
pages* 89,90,113 and 114 received by this Authority on 02 February 2006 (02.02.2006)
pages* NONE received by this Authority on _____

☒ the drawings:
pages 1,2 and 4-13 as originally filed/furnished
pages* 3 received by this Authority on 02 February 2006 (02.02.2006)
pages* NONE received by this Authority on _____

☐ a sequence listing and/or any related table(s) - see Supplemental Box Relating to Sequence Listing.

3. ☐ The amendments have resulted in the cancellation of:

☐ the description, pages _____
☐ the claims, Nos _____
☐ the drawings, sheets/figs _____
☐ the sequence listing (*specify*): _____
☐ any table(s) related to the sequence listing (*specify*): _____

4. ☐ This report has been established as if (some of) the amendments annexed to this report and listed below had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).

☐ the description, pages _____
☐ the claims, Nos _____
☐ the drawings, sheets/figs _____
☐ the sequence listing (*specify*): _____
☐ any table(s) related to the sequence listing (*specify*): _____

* *If item 4 applies, some or all of those sheets may be marked "superseded."*

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

International application No.
PCT/US05/18412

Box No. V Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Claims 1-64	YES
	Claims NONE	NO
Inventive Step (IS)	Claims 1-64	YES
	Claims NONE	NO
Industrial Applicability (IA)	Claims 1-64	YES
	Claims NONE	NO

2. Citations and Explanations (Rule 70.7)

Claims 1-64 meet the criteria set out in PCT Article 33(2)-(3), because the prior art does not teach or fairly suggest the theramutein modulators as claimed by the Applicant. The prior art does not teach or fairly suggest the compounds and the methods using the theramutein modulators claimed by Applicant.

Claims 1-64 meet the criteria set out in PCT Article 33(4), and thus have industrial applicability because the subject matter claimed can be made or used in industry.

subjects by providing alternative drug substances that will be effective against said theramutein.

1. The invention provides a method of determining whether a chemical agent is at least as effective a modulator of a theramutein in a cell as a known substance is a modulator of a corresponding prototheramutein. One embodiment of the method involves contacting a control cell that expresses the prototheramutein and is capable of exhibiting a responsive phenotypic characteristic (linked to the functioning of the prototheramutein in the cell) with the known modulator of the prototheramutein, contacting a test cell that expresses the theramutein and is also capable of exhibiting the responsive phenotypic characteristic (linked to the functioning of the theramutein in the cell) with the chemical agent, and comparing the response of the treated test cell with the response of the treated control cell; to determine that the chemical agent is at least as effective a modulator of the theramutein as the known substance is a modulator of the prototheramutein. In certain other embodiments, one type of control cell may not express the prototheramutein at all. In other embodiments, the control cell may express about the same amount of the prototheramutein as the test cell expresses of the theramutein. In still other embodiments, the control cell may be capable of exhibiting the responsive phenotypic characteristic to about the same extent as the test cell under certain conditions.

2. Theramuteins of the invention that are of particular interest are those involved in regulatory function, such as enzymes, protein kinases, tyrosine kinases, receptor tyrosine kinases, serine threoninin protein kinases, dual specificity protein kinases, proteases, matrix metalloproteinases, phosphatases, cell cycle control proteins, docking proteins such as the IRS family members, cell-surface receptors, G-proteins, ion channels, DNA- and RNA-binding proteins, polymerases, and the like. No limitation is intended on the type of theramutein that may be used in the invention. At the present time, three theramuteins are known: BCR-ABL, c-Kit, and EGFR.

3. Any responsive phenotypic characteristic that can be linked to the presence of the theramutein (or prototheramutein) in the cell can be employed for use in the method, including, for example, growth or culture properties, the phosphorylation state (or other modification) of a substrate of the theramutein, and any type of transient characteristic of the cell, as will be defined and discussed in detail

AMENDED SHEET

DESCRIPTION OF THE FIGURES

[0017] Figure 1 shows the effect on growth and viability of different concentrations of Compound 2 (C2) for non-transformed vector control Ba/F3 cells (which are IL-3 dependent) as well as Ba/F3 cells expressing the "wild type" $p210^{Bcr\text{-}Abl}$ (designated $p210^{Bcr\text{-}Abl\text{-}wt}$), and Ba/F3 cells expressing the $p210^{Bcr\text{-}Abl\text{-}T315I}$ drug resistant mutant. Cell counts and viability were determined on an automated cell counter as discussed in detail in the specification. Cell counts are shown by the solid color bars; cell viability is shown by the hashed bars. Note that STI-571 potently inhibits growth of the P210 cell line (grey bar) whereas it is unable to inhibit the growth of the T315I cell line (white bar) even at 10 μM concentration. 500 nM C2 shows the largest specificity gap within this dose-response series. Compare STI-571 at 10 μM to C2 at 500 nM on the T315I cell line (white bars). Abbreviations: DMSO: dimethylsulfoxide (solvent used for drug dissolution).

[0018] Figure 2 shows the effect on growth and viability of different concentrations of Compound 6 (C6) for non-transformed vector control Ba/F3 cells as well as Ba/F3 cells expressing the $p210^{Bcr\text{-}Abl\text{-}T315I}$ drug resistant mutant. All other details are as per Fig. 1.

[0019] Figure 3 shows various determinations of the specificity gap by comparing the effects of various compounds identified in the screen in terms of their effects on the prototheramutein- and theramutein-expressing cell lines. Compound 3 (C3) shows the best example of the ability of the method to identify a compound that exerts an even greater effect on the theramutein than on its corresponding prototheramutein. (Panel E). Panel A: control DMSO treatments; B: negative heterologous specificity gap; C: slightly positive heterologous specificity gap; D: large positive homologous specificity gap; E: positive heterologous specificity gap. See text for explanations.

[0020] Figure 4 shows an autoradiograph of recombinant P210 Bcr-Abl wild type and T315I mutant kinase domains assayed for autophosphorylation activity. 200 ng of protein were preincubated with test substances for 10 minutes under standard autophosphoryation reaction conditions and then radiolabelled ATP was added and the reactions proceeded for 30 minutes at 30°C, after which the samples were separated by SDS-PAGE. The gels were silver-stained, dried down under vacuum and exposed to X-ray film. Note that whereas 10 μM STI 571 is effective against wild type P210 Bcr-Abl, it is virtually ineffective against the T315I kinase domain, even at concentrations up to 100 μM. C2 and C6 are the best two

Y is selected from a chemical bond, O, NR^0,

R^6 is selected from alkyl, cycloalkyl, alkenyl, alkynyl, aralkyl, aryl, and a heterocyclic ring;

Z is a hydrocarbon chain having from 1 to 4 carbon atoms, and optionally substituted with one or more of halo, alkyl, cycloalkyl, alkenyl, alkynyl, aralkyl, CO_2R^0, $C(O)R^0$, $C(O)N(R^0)_2$, CN, CF_3, $N(R^0)_2$, NO_2, and OR^0;

R^7 is H or is selected from aryl and a heterocyclic ring;

each R^0 is independently selected from H, alkyl, cycloalkyl, aralkyl, aryl and a heterocyclic ring;

a is 1 or 2;

b is 0 or 1;

c is 1 or 2;

d is 0 or 1;

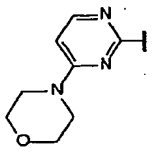
e is 1 or 2; and

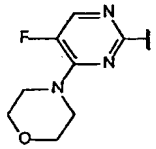
f is 0 or 1.

[0041] An important component and conceptual teaching of the Invention described herein is that neither the R^2 nor the R^3 positions of the compounds of this invention are members of any aromatic or non-aromatic ring structure. We have discovered that compounds having the R^2 and/or the R^3 positions as members of any aromatic or non-aromatic ring structure do not effectively inhibit the T315I theramutein, whereas the compounds of the invention that lack such a ring component at these positions, in addition to having other preferred chemical groups, are potent inhibitors of the T315I theramutein.

[0042] In preferred embodiments of the invention, ring A is an aromatic ring.

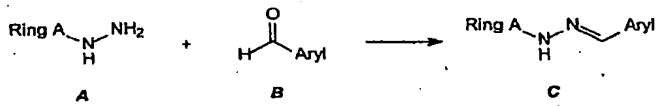
[0043] In preferred embodiments of the invention, X^1 or X^2 is N. In another preferred embodiment, both X^1 and X^2 are N. In particularly preferred embodiments of the invention Ring A is a pyridine ring or a pyrimidine ring. In still further preferred embodiments, Ring A is selected from the structures provided below:





chemistry would be readily familiar with the procedures and techniques necessary to accomplish the synthetic approaches given below.

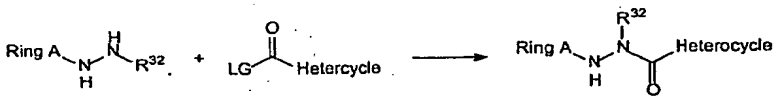
[0072] Embodiments wherein R^2 = NH, R^3 = N, R^4 = CH, and R^5 = -aryl may be prepared by reaction of an appropriate hydrazine compound, such as ***A***, and an appropriate aldehyde, such as ***B***, under conditions similar to those described on p. 562 of Gineinah, *et al.* (Arch. Pharm. Med. Chem. **2002**, 335, 556-562).



A ***B*** ***C***

For example, heating ***A*** with 1.1 equivalents of ***B*** for 1 to 24 hours in a protic solvent such as a C_1 to C_6 alcohol, followed by cooling and collection of the precipitate, would afford ***C***. Alternatively, product ***C*** may be isolated by evaporation of the solvent and purification by chromatography using silica gel, alumina, or C_4 to C_{18} reverse phase medium. Similar methodology would be applicable in the cases where "Aryl" is replaced by other groups defined under R^5.

[0073] Embodiments wherein R^2 = NH, R^3 = NR^{32}, R^4 = C(O), and R^5 = a heterocyclic ring may be prepared by reaction of an appropriate hydrazine compound, such as ***D***, and an activated carboxylic acid such as ***E***, wherein LG is a leaving group such as halo, 1-oxybenztriazole, pentafluorophenoxy, *p*-nitrophenoxy, or the like, or Compound ***E*** may also be a symmetrical carboxylic acid anhydride, whereby conditions similar to those described on p. 408 of Nair and Mehta (Indian J. Chem. **1967** 5, 403-408) may be used.

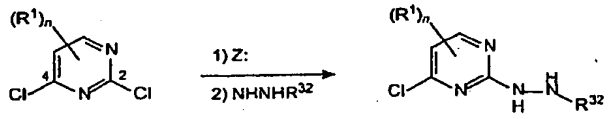


D ***E*** ***F***

For example, treatment of ***D*** with an active ester such as Heterocycle-C(O)-OC_6F_5 in an inert solvent such as dichloromethane, 1,2-dichloroethane, or N,N-dimethylformamide, optionally in the presence of a base such as pyridine or another tertiary amine, and optionally in the presence of a catalyst such as 4-*N*,*N*-dimethylaminopyridine, at an appropriate temperature

ranging from 0° C to the boiling point of the solvent, would afford ***F***, which may be isolated by evaporation of the solvent followed by chromatography using silica gel, alumina, or C_4 to C_{18} reverse phase medium. The above active ester example of ***E*** would be readily prepared from the corresponding carboxylic acid and pentafluorophenol using a carbodiimide such as dicyclohexylcarbodiimide as a condensing agent. Similar methodology would be applicable in the cases where "Heterocycle" is replaced by other groups defined under R^5.

[0074] Precursors such as ***A*** and ***D*** may be prepared by reaction of an appropriate nucleophile, for example, a hydrazine derivative, with a heteroaromatic compound bearing a halo substituent at a position adjacent to a nitrogen atom. For example, using methods analogous to those described by Wu, *et al.* (J. Heterocyclic Chem. **1990**, 27, 1559-1563), Breshears, *et al.* (J. Am. Chem. Soc. **1959**, 81, 3789-3792), or Gineinah, *et al.* (Arch. Pharm. Med. Chem. **2002**, 335, 556-562), examples of compounds ***A*** and ***D*** may be prepared starting from, for example, a 2,4-dihalopyrimidine derivative, many of which are commercially available or are otherwise readily prepared by one skilled in the art. Thus, treatment of an appropriate 2,4-dihalopyrimidine derivative ***G*** with an amine or other nucleophile (Z), optionally in the presence of an added base, selectively displaces the 4-halo substituent on the pyrimidine ring. Subsequent treatment of the product with a second nucleophilic reagent such as hydrazine or a hydrazine derivative, optionally in a solvent such as a C_1 to C_6 alcohol and optionally in the presence of an added base, displaces the 2-halo substituent on the pyrimidine ring, to afford compounds that are examples of structures ***A*** and ***D*** above.



G

[0075] Embodiments wherein R^2 is $-NR^{22}$ and R^3 is $-C(=R^{33})$ can be synthesized by methods such as the following, or straightforward modifications thereof. The synthesis may be conducted starting from an appropriate ring A derivative ***J*** that bears a leaving group (LG) adjacent to the requisite ring nitrogen. Structure ***G*** above and the product of reaction of structure ***G*** with nucleophile Z, as illustrated above, are examples of such appropriate Ring A derivatives ***J***. Suitable LG' groups are halo, alkylthio, alkylsulfonyl, alkylsulfonate or

activator may be partial or complete. Likewise, as used herein, the terms "antagonist" and "inhibitor" of a protein are used interchangeably. An inhibitor (antagonist) is limited to a substance that binds to and inhibits the functioning of a given protein. To state that a substance "inhibit(s)" a protein means the substance binds to the protein and reduce(s) the protein's activity in the cell without materially reducing the amount of the protein in the cell. Similarly, to state that a substance "activate(s)" a protein, such as a prototheramutein or theramutein, is to state that the substance increased the defined function of the protein in the cell without substantially altering the level of the protein in the cell. Unless explicitly stated otherwise, an "inhibitor", an "antagonist" and an "inhibitor of a protein" are also synonymous. The inhibition by an inhibitor may be partial or complete. A modulator is an activator or an inhibitor. By way of example, an "activator of $PKC_{\beta 1}$" should be construed to mean a substance that binds to and activates $PKC_{\beta 1}$. Similarly, an "inhibitor of $p210^{Bcr\text{-}Abl}$" is a substance that binds to and inhibits the functioning of $p210^{Bcr\text{-}Abl}$. To state that a substance "inhibits a protein" requires that the substance bind to the protein in order to exert its inhibitory effect. Similarly, to state that a substance "activates protein X" is to state that the substance binds to and activates protein X. The terms "bind(s)," "binding," and "binds to" have their ordinary meanings in the field of biochemistry in terms of describing the interaction between two substances (*e.g.*, enzyme-substrate, protein-DNA, receptor-ligand, etc.). As used herein, the term "binds to" is synonymous with "interacts with" in the context of discussing the relationship between a substance and its corresponding target protein. As used herein, to state that a substance "acts on" a protein, "affects" a protein, "exerts its effect on" a protein, etc., and all such related terms uniformly mean (as the skilled investigator is well aware) that said substance activates or inhibits said protein.

[0094] The concept of inhibition or activation of a mutated form of an endogenous protein to a greater extent than the corresponding non-mutated counterpart protein is defined for the first time and referred to herein as a positive "***specificity gap***." In general terms, *and using an inhibitor case as an example*, the ***specificity gap*** refers to the difference between the ability of a given substance, under comparable conditions to inhibit the theramutein in a cell-based assay system as compared to either:

a) the ability of the same substance under comparable conditions to inhibit the prototheramutein, or

b) the ability of a second substance (usually a known inhibitor of the prototheramutein) to inhibit the theramutein under comparable conditions, or

c) the ability of the second substance to inhibit the prototheramutein under comparable conditions.

[0095] When the comparison is made between the effects of two distinct substances (tested individually) on the theramutein alone, the result is termed a ***homologous specificity gap*** determination.

[0096] Alternatively, when a comparison is made between the effects of two distinct substances (generally, but not always), one of which is tested on the theramutein and the other on the prototheramutein, respectively, the result is termed a ***heterologous specificity gap*** **(SG)** determination. Thus, (a) and (c) as given above are examples of heterologous specificity gap (SG) determinations (although (a) uses the same substance in both instances), whereas (b) is an example of a homologous specificity gap determination.

[0100] Reference to Figure 3 is informative in understanding and elucidating these concepts.

[0101] Analogous issues apply when the case concerns an activator. It will be immediately obvious to the skilled artisan that the term "comparable conditions" includes testing two different compounds, for example, at the same concentration (such as comparing two closely related compounds to determine relative potency), or by comparing the effects of two different compounds tested at their respective IC_{50} values on the corresponding prototheramutein and theramutein. The skilled investigator will easily recognize other useful variations and comparable conditions.

[0102] Thus, in one embodiment of the application of this approach, substances that are more effective against a theramutein have a "positive specificity gap." A "zero, null or no" specificity gap indicates that there is no significant measurable difference between the effect of a substance on the theramutein as compared to its effect on the prototheramutein (however such compounds may be quite useful in their ability to inhibit or activate both a theramutein and its corresponding prototheramutein), and a "negative specificity gap" indicates a substance that at a given concentration is less effective against the given theramutein than against a form of the corresponding prototheramutein or other comparative form of the theramutein (such as one that may harbor a different mutation). The latter category is generally of lesser interest than the former categories of compounds, except in the

to STI-571. Hence purely and simply, the Huron methodology failed to identify an effective inhibitor of the $p210^{Bcr\text{-}Abl T315I}$ theramutein.

[0109] Indeed, prior to the disclosure of this invention, including both the detailed methodology described for the first time herein as well as the compositions provided herein, *no one anywhere in the world* has been successful in identifying a chemical agent, let alone a methodology that is capable of identifying a chemical agent that effectively inhibits the $p210^{Bcr\text{-}Abl T315I}$ theramutein to an equal or greater extent than STI-571 is able to do with respect to the wild type $p210^{Bcr\text{-}Abl}$ protein. (See Shah et al., Science, July, 2004; O'Hare et al., Blood, 2004; Tipping et al., Leukemia, 2004; Weisberg et al., Leukemia, 2004).

[0110] It cannot be overemphasized that such compounds would be immensely useful, because at the present time there is no alternative for patients who progress to $p210^{Bcr\text{-}Abl\text{-}T315I}$ theramutein-mediated imatinib mesylate-resistant status. *Once patients develop such resistance, there is no other effective alternative treatment available, and death is certain. The method described herein provides the first reported approach to identify, pharmacologically characterize and chemically synthesize effective inhibitors* of the $p210^{Bcr\text{-}Abl\text{-}T315I}$ *theramutein. Moreover, the skilled investigator will immediately recognize the applicability and generalizability of this approach to any highly drug-resistant theramutein.*

[0111] In the present invention, a test cell is used that displays a carefully selected phenotypic characteristic (as defined below) which is linked to the presence and functional activity of the particular theramutein-of-interest (TOI) in the cell under appropriate conditions. This should be qualitatively the same as the phenotypic characteristic displayed by a cell that expresses the prototheramutein. A phenotypic characteristic (i.e. a non-genotypic characteristic of the cell) is a property which is observed (measured), selected and/or defined for subsequent use in an assay method as described herein. Expression of the phenotypic characteristic is responsive to the total activity of the theramutein in the cell, and is a result of the absolute amount of the theramutein and its specific activity. Often, the phenotypic characteristic is observable as a result of elevated levels of theramutein activity and is not apparent in cells that express low amounts of the theramutein or low amounts of its corresponding prototheramutein. Further, it can often be demonstrated that the phenotypic characteristic is modulated by modulating the specific activity of the theramutein with an inhibitor or activator of the theramutein , although this is not always the case since an inhibitor or activator of the TOI may not always be available at the time the skilled

selected protein. Such a responsive phenotypic characteristic is referred to herein as a "phenoresponse."

[0117] Though not always necessary, it will often be advantageous to employ cells that express high levels of the theramutein, and to select a phenotypic characteristic that results from overexpression of the theramutein. This is because phenotypic characteristics linked to the functioning of the theramutein generally become more distinguishable (easier to measure) as a theramutein is overexpressed to a greater extent. Further, phenoresponses that are observed in response to modulators of the theramutein are often amplified as the functional level of the theramutein is increased. Expressed another way, the selected phenoresponse observed in cells that overexpress the theramutein is particularly sensitive to modulators of the theramutein.

[0118] Preferably, the theramutein is stably expressed in a test cell. Stable expression results in a level of the theramutein in the cell that remains relatively unchanged during the course of an assay. For example, stimulation or activation of a component of a signaling pathway may be followed by a refractory period during which signaling is inhibited due to down-regulation of the component. For theramuteins of the invention, such down-regulation is usually sufficiently overcome by artificially overexpressing the theramutein. Expressed another way, the expression is sufficiently maintained that changes in a phenotypic characteristic that are observed during the course of an assay are due primarily to inhibition or activation of the theramutein, rather than a change in its level, even if down-modulation of the theramutein subsequently occurs. For these reasons, although stable expression of the theramutein is preferred, transfection followed by transient expression of the theramutein may be employed provided that the selected phenotypic characteristic is measurable and the duration of the assay system is short relative to the progressive decline in the levels of the transiently expressed theramutein which is to be expected in such systems over time. For these reasons, stably expressing cell lines are preferred (U.S. Patent No. 4,980,281).

[0119] A preferred drug screening method of the present invention involves the following:

[0120] 1) Identification of a theramutein for which a novel inhibitor or activator is desired. Identification of an appropriate theramutein may be performed using standard techniques (See, Gorre et al., Science, 2001; see also PCT/US02/18729). Briefly, patients that have been given a course of a therapeutically effective treatment using an activator or

methodology. (Gorre et al., 2001; Housey et al., 1988). In one embodiment, overexpression results in a level of the theramutein that is at least about 3 times the amount of the protein usually present in a cell. Alternatively, the amount is at least about 10 times the amount usually present in a cell. In another embodiment, the amount is at least about 20 times or more preferably at least about 50 times the amount usually present in a cell.

[0122] 3) Provision of a control cell that expresses the prototheramutein corresponding to the theramutein of interest. As some of the muteins that are described herein are also enzymes, they usually retain catalytic activity, and therefore the control cell usually displays substantially the same phenotypic characteristic as the test cell. The phenotypic characteristic need not be quantitatively alike in both cells, however. For example, a mutation that leads to reactivation of the prototheramutein may also increase, decrease, or otherwise affect its specific activity with respect to one or more of its substrates in the cell. As a result, it may exhibit the selected phenotypic characteristic to a greater or lesser extent. Accordingly, it may be desirable in some cases to adjust expression of either or both of the prototheramutein and the theramutein such that test and control cells exhibit the phenotypic characteristic to approximately the same degree. This may be done, for example, by expressing the proteins from promoters whose activity can be adjusted by adjusting the amount of inducer present, all using standard methodology (see, for example, Sambrook et al. 1989 & 2001).

[0123] It will be obvious to one of ordinary skill in the art that a properly defined phenoresponse may be *quantitatively* different between the prototheramutein- and the theramutein-expressing cell lines as a result of differences in the specific activity (if any) between the theramutein and its corresponding prototheramutein. Theramutein-inducing mutations may increase or decrease the specific activity of said theramutein relative to the corresponding prototheramutein. When comparing a theramutein expressing cell line with a prototheramutein expressing cell line, it is preferable that the selected phenoresponse is qualitatively the same in both cell types. Thus, the skilled investigator may choose to normalize the activity of the theramutein-expressing cell line to that of the prototheramutein-expressing cell line, or vice versa. Such normalization methods are standard in the art. See, for example, Bolstad et al. (2003).

[0124] Alternatively, the skilled investigator may also wish to use unmodified host cells or host cells harboring the expression vector only as control cells for certain

experimental procedures. (The host cells are the cells into which an expression vector encoding the theramutein was introduced in order to generate the test cells.) This may be the case where the investigator is only interested in identifying a specific inhibitor or activator of the theramutein of interest, irrespective of whether or not said compound is also active effective the prototheramutein of interest (pTOI).

[0125] 4) The test and control cells are then maintained or propagated (although not necessarily at the same time) in growth media (or even in intact animals) under suitable conditions such that the phenoresponse may be expressed and assayed. Control cells that are expressing the prototheramutein may be treated with a known modulator of the prototheramutein, or with a test substance, and test cells are treated with test compounds to determine whether they are active against the theramutein, as measured by the ability of said substances to modulate the phenoresponse in the expected manner. Alternatively, control cells not expressing the prototheramutein may also be substituted, depending upon the particular phenoresponse that the skilled investigator has chosen for study. Substances may then be assayed on the test cells and, optionally, on the control cells at the same time, or at another time, and the results compared.

[0126] In one embodiment of the invention, substances that are active with regard to the test cells can be rapidly identified by their ability to modulate the phenoresponse of the test cells in the same manner as, for example, the known modulator of the prototheramutein alters the phenoresponse of prototheramutein-expressing control cells. In another embodiment, active substances may be identified by their ability to modulate the activity of the theramutein in the test cells while having little or no effect on the unmodified (prototheramutein and/or theramutein non-expressing) control cells. The skilled investigator will readily appreciate the many variations of this approach that may be utilized to identify, for example, modulators that are more effective against the theramutein, or that are equally effective against both the prototheramutein and one or more corresponding specific theramuteins.

[0127] Other phenoresponses can be observed and/or measured and include, for example, detection of substrates of the prototheramutein, and detection of gene expression changes that are regulated by the activity of the theramutein. In the simplest terms, any characteristic of the cell that the skilled investigator has previously correlated with the functional activity of the theramutein may be suitable for use with such methods. However,

commencing the anti-neoplastic agent therapy. For example, the theramutein inhibitor can be administered between 1 and 30 days, preferably 3 and 20 days, more preferably between 5 and 12 days before commencing radiation therapy. In a preferred embodiment of the invention, chemotherapy is administered prior to, concurrently with or, more preferably, subsequent to antibody therapy.

[0140] In the present invention, any suitable method or route can be used to administer theramutein inhibitors of the invention, and optionally, to co-administer anti-neoplastic agents and/or antagonists of other receptors. The anti-neoplastic agent regimens utilized according to the invention, include any regimen believed to be optimally suitable for the treatment of the patient's neoplastic condition. Different malignancies can require use of specific anti-tumor antibodies and specific anti-neoplastic agents, which will be determined on a patient to patient basis. Routes of administration include, for example, oral, intravenous, intraperitoneal, subcutaneous, or intramuscular administration. The dose of antagonist administered depends on numerous factors, including, for example, the type of antagonists, the type and severity of the tumor being treated and the route of administration of the antagonists. It should be emphasized, however, that the present invention is not limited to any particular method or route of administration.

[0141] Suitable carriers include, for example, one or more of water, saline, phosphate buffered saline, dextrose, glycerol, ethanol and the like, as well as combinations thereof. Carriers can further comprise minor amounts of auxiliary substances, such as wetting or emulsifying agents, preservatives or buffers, which enhance the shelf life or effectiveness of the theramutein modulator as the active ingredient. The compositions can, as is well known in the art, be formulated so as to provide quick, sustained or delayed release of the active ingredient after administration to the mammal.

[0142] The compositions of this invention can be in a variety of forms. These include, for example, solid, semi-solid and liquid dosage forms, such as tablets, pills, powders, liquid solutions, dispersions or suspensions, liposomes, suppositories, injectable and infusible solutions. The preferred form depends on the intended mode of administration and therapeutic application.

[0143] Such compositions of the present invention are prepared in a manner well known in the pharmaceutical art. In making the composition the active ingredient will usually be mixed with a carrier, or diluted by a carrier and/or enclosed within a carrier which

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R^{45} is selected from -Y"-R^{19};

Y" is selected from a chemical bond, O, NR^0-, and a hydrocarbon chain having from 1 to 4 carbon atoms, and optionally substituted with one or more of halo, alkyl, cycloalkyl, alkenyl, alkynyl, aralkyl, CO_2R^0, $C(O)R^0$, $C(O)N(R^0)_2$, CN, CF_3, $N(R^0)_2$, NO_2, and OR^0;

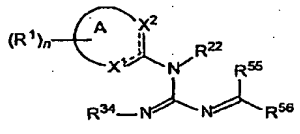
R^{19} is selected from the group consisting of H, alkyl, cycloalkyl, alkenyl, alkynyl, aralkyl, CF_3, aryl, and a heterocyclic ring; and

each R^0 is independently selected from H, alkyl, cycloalkyl, aralkyl, aryl and a heterocyclic ring.

24. The method of claim 23 wherein X^1 is N.

25. The method of claim 24 wherein X^2 is N.

26. The method of claim 1; comprising administering a therapeutically effective amount of a compound having the formula V



(V)

wherein:

ring A is a 5-, 6-, or 7- membered ring or a 7- to 12-membered fused bicyclic ring;

X^1 is selected from N, N-R^0 or C-R^1;

X^2 is selected from N, N-R^0 or C-R^1;

the dotted lines represent optional double bonds;

each R^1 is independently selected from the group consisting of H, alkyl, cycloalkyl, alkenyl, alkynyl, aralkyl, CN, CF_3, NO_2, OR^{11}, $-(CH_2)_pC(O)(CH_2)_qR^{11}$, $-(CH_2)_pC(O)N(R^{12})(R^{13})$, $-(CH_2)_pC(O)O(CH_2)_qR^{11}$, $-(CH_2)_pN(R^{11})C(O)R^{11}$, $-(CH_2)_pN(R^{12})(R^{13})$, $-N(R^{11})SO_2R^{11}$, $-OC(O)N(R^{12})(R^{13})$, $-SO_2N(R^{12})(R^{13})$, halo, aryl, and a heterocyclic ring, and additionally or alternatively, two R^1 groups on adjacent ring atoms form a 5- or 6-membered fused ring which contains from 0 to 3 heteroatoms;

n is 0 to 6,

each R^{11} is independently selected from H, alkyl, cycloalkyl, alkenyl, alkynyl, aralkyl, aryl, and a heterocyclic ring;

each R^{12} and R^{13} are independently selected from H, alkyl, cycloalkyl, alkenyl, alkynyl, aralkyl, aryl, and a heterocyclic ring; or R^{12} and R^{13} may be taken

together with the nitrogen to which they are attached form a 5- to 7- membered ring which may optionally contain a further heteroatom;

p is 0 to 4;

q is 0 to 4;

R^{22} is selected from H and C_{1-3} alkyl;

R^{34} is selected from H, NO_2, CN, alkyl, cycloalkyl, alkenyl, alkynyl, aralkyl, aryl and a heterocyclic ring;

R^{55} is selected from H, alkyl, cycloalkyl, alkenyl, alkynyl, aralkyl, aryl, and a heterocyclic ring;

R^{56} is selected from -Y"-R^{19};

Y" is selected from a chemical bond, O, NR^0-, and a hydrocarbon chain having from 1 to 4 carbon atoms, and optionally substituted with one or more of halo, alkyl, cycloalkyl, alkenyl, alkynyl, aralkyl, CO_2R^0, $C(O)R^0$, $C(O)N(R^0)_2$, CN, CF_3, $N(R^0)_2$, NO_2, and OR^0;

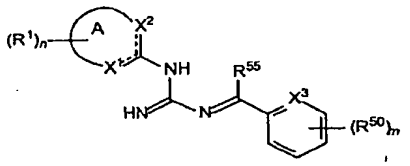
R^{19} is selected from the group consisting of H, alkyl, cycloalkyl, alkenyl, alkynyl, aralkyl, CF_3, aryl, and a heterocyclic ring; and

each R^0 is independently selected from H, alkyl, cycloalkyl, aralkyl, aryl and a heterocyclic ring.

27. The method of claim 26 wherein X^1 is N.

28. The method of claim 27 wherein X^2 is N.

29. The method of claim 1, comprising administering a therapeutically effective amount of a compound having the formula V_a



(V_a)

wherein:

ring A is a 5-, 6-, or 7- membered ring or a 7- to 12-membered fused bicyclic ring;

X^1 is selected from N, N-R^0 or C-R^1;

each R^0 is independently selected from H, alkyl, cycloalkyl, aralkyl, aryl and a heterocyclic ring;

h is 0 to 4;

i is 0 to 4; and

j is 0 to 4.

52. A method for determining whether a substance is an inhibitor or an activator of a theramutein which is capable of eliciting a detectable phenoresponse, which comprises:

a) incubating a first cell which expresses the theramutein at a substantially constant level with the substance;

b) incubating a second cell which expresses a corresponding prototheramutein at a substantially constant level with a known inhibitor or activator of the prototheramutein;

c) comparing a phenoresponse of the second cell to the known inhibitor or activator of the prototheramutein to the phenoresponse of the first cell to the substance; and

d) determining that the phenoresponse of the first cell is inhibited or activated to at least the same degree as the phenoresponse of the second cell is inhibited or activated by the known inhibitor or activator of the prototheramutein, thereby identifying the substance as an inhibitor or an activator of the theramutein.

53. The method of Claim 52, wherein the phenoresponse of the cell expressing the theramutein to the substance is greater than the phenoresponse of the cell expressing the prototheramutein to the known inhibitor or activator of the theramutein.

54. A method for determining whether a substance is a specific inhibitor or specific activator of a theramutein, which comprises:

a) providing a test cell which expresses the theramutein and which gives rise to a detectable phenoresponse;

b) treating the test cell with the substance;

c) examining the treated cell to determine whether the phenoresponse is modulated by treatment with the substance.

55. The method of Claim 52 or 54, wherein the theramutein or prototheramutein is a component of a signal transduction cascade.

56. The method of Claim 52 or 54, wherein the theramutein or prototheramutein is an enzyme.

57. The method of Claim 52 or 54, wherein the theramutein or prototheramutein is a protein kinase.

58. The method of Claim 52 or 54, wherein the theramutein or prototheramutein is a tyrosine kinase.

59. The method of Claim 52 or 54, wherein the theramutein or prototheramutein is a receptor tyrosine kinase.

60. The method of Claim 52 or 54, wherein the or prototheramutein is $p210^{Bcr\text{-}Abl}$.

61. The method of Claim 52 or 54, wherein the or prototheramutein is the T315I mutant of $p210^{Bcr\text{-}Abl}$.

62. The method of Claim 52 or 54, wherein the phenoresponse is a change in a cultural, morphological, or transient characteristic of the cell.

63. The method of Claim 52 or 54, wherein the phenoresponse includes phosphorylation of an intracellular substrate of the theramutein.

64. The method of Claim 52 or 54, wherein the phenoresponse is detected on a subcellular fraction of the cell.